ANTI ULCER EFFECT OF ALANGIUM SALVIFOLIUM ETHANOLIC LEAF EXTRACT ON GASTRIC LESION INDUCED BY ETHANOL IN RATS


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ABSTRACT
Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. Alangium salvifolium, belongs to the family of Alanginaceae. Based on the ethno pharmacological literatures, the plant Alangium salvifolium has been selected to prove its anti ulcer property on experimental animal models. The phytoconstituents present in the plant were extracted by using different solvents of increasing polarity like n-hexane, chloroform, ethyl acetate, acetone, ethanol and water. The phytoconstituents were identified by various chemical tests which showed the presence of various phytoconstituents. The ethanolic extract of leaves of Alangium salvifolium was selected for the pharmacological screening and evaluated using mice for its toxicity. The LD50 was calculated by using Karber’s method. The ED50 value was found to be 150 mg/kg body weight. By performing anti ulcer activity on male Wistar rats and screened pharmacologically for the anti ulcer activity. From our findings it can be concluded that the ethanolic extract of Alangium salvifolium has a significant anti ulcer activity at 400mg/kg and 800mg/kg dose. The results were comparable with that of standard and control groups.

Key words: Herbalism, Ethanol induced ulcers, Anti ulcer activity, Alangium salvifolium.

INTRODUCTION
Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. Herbalism is also known as botanical medicine, medical herbology, herbal medicine, , herbology, and phyotherapy. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts. Ulcer can be a life-threatening condition. The danger is in perforation of the gut wall, which can result in death. The symptoms of gastric ulcer are also similar to gastric cancer, so ulcer patients should be under the care of a physician. The most commonly prescribed ulcer drugs are cimetidine and ranitidine 1. The increased popularity in herbal remedy for anti ulcer therapy can be attributing to the belief that the herbal drugs provide some benefit over allopathic medicine including low toxicity. Every system of medicine has its own anti-ulcer products. Allopathy also has many developed products in this regard. But all such products also have a long range of side effects. So, in order to avoid such side effects of allopathic drugs, the world is looking towards the use of herbal drugs 2.

Alangium salvifolium, belongs to the family of Alanginaceae. It is well distributed in India; the leaf is entire margin, simple, petiolate, lanceolate, narrowly oblong or ovate, base rounded or acute pubescent, alternate, reticulate venation, up to 15 cm long, long petioled, apex obtuse, glabrescent 3. The leaves can be used as hypoglycemic, anti diabetic, anti protozoal, anti cholinesterase, antispasmodic and as an anti arthritic. Alangium salvifolium has been used traditionally for its various therapeutic properties like laxative, astrigent, pungent, anthelmintic, purgative, emetic, anti protozoal, hypoglycemic activity, anti diabetic and for anti ulcer activity 4. A recent study on the in vitro evaluation of anti fungal activity 5, anti diabetic activity 5, antioxidant and anti microbial activity 6, cardiac activity 7 and anti fertility activity 8. Based on the ethno pharmacological literatures, the plant Alangium salvifolium has been selected to screen for its anti ulcer property on experimental animal models.

MATERIALS AND METHODS
Collection of plant material
The plant was collected nearby foot hill of Rayachoti, Kadapa District of Andhra Pradesh in the month of November, 2010. The collected species were authenticated by Botanist, Dr. K. Madhava Satty, Asst. Professor, Department of Botany, S.V. University, Tirupati. Leaves were separated carefully and made free from earthy matter and other plant parts, and were subjected for shade drying. Then they were powdered using mechanical grinder and the crude powder was employed for extraction.

Preparation of extract
The extraction was carried out with various solvents by hot percolation method. The powdered leaves were evenly packed in the soxhlet apparatus. Then it was extracted with various solvents from non-polar to polar i.e. in the order, n-hexane, chloroform, ethyl acetate, acetone, ethanol and water successively. The Soxhlet is then equipped with a condenser. After each extraction, the extracts were filtered though whatmann filter paper to remove any impurities if necessary. Then they were concentrated in desiccators to remove any excessive moisture 9. Then the concentrated extracts were transferred in to a beaker and the remaining solvent was evaporated on a water bath. Then they were cooled and placed in desiccators to remove any excessive moisture 9. Then the extracts were packed in air tight containers for their further use in the phytochemical screening and pharmacological studies. The extractive values of various extracts were given in the table no – 1.

Table 1: Shows the Extractive Values of Alangium salvifolium

<table>
<thead>
<tr>
<th>S. No</th>
<th>Type of Extract</th>
<th>Percentage yield (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>n – hexane</td>
<td>4.46</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform</td>
<td>3.34</td>
</tr>
<tr>
<td>3.</td>
<td>Ethyl acetate</td>
<td>2.02</td>
</tr>
<tr>
<td>4.</td>
<td>Acetone</td>
<td>3.64</td>
</tr>
<tr>
<td>5.</td>
<td>Ethanol</td>
<td>3.64</td>
</tr>
</tbody>
</table>

Identification Of Phyto Constituents
The preliminary phytochemical studies were performed using standard procedures to identify the phyto constituents present in the extracts 10. The results were tabulated in Table No. 2. As the ethanolic extract was rich of various phytoconstituents, it was selected for the pharmacological study.

PHARMACOLOGICAL STUDIES
Acute oral toxicity studies
The mice weighing about 20-40 gm were fasted over night. Then they were weighed and divided in to four groups of four animals in each. The ethanolic extract of Alangium salvifolium was dissolved in tween 80 and then administered to mice in various doses i.e. 50mg/kg, 100mg/kg, 200mg/kg, 500mg/kg, 1000mg/kg by oral route. After the administration of the crude extract, the animals
were observed continuously for the first two to four hours for the death due to acute toxicity. Then the results of the LD50 study performed on mice were expressed using Karber's method. The results obtained were expressed in the table no. 3.

From the table it can be concluded that there is no mortality and toxicity symptoms for the ethanolic extract. So the dose was optimized up to 1500mg/kg.

Table 2: Shows the Identification of Phyto Constituents Of Alangium Salvifolium

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Alkaloids</th>
<th>Carbohydrates</th>
<th>Steroids &amp; terpenoids</th>
<th>Tannins</th>
<th>Phenolic compounds</th>
<th>Proteins amino acids</th>
<th>Flavonoids</th>
<th>Glycosides</th>
<th>Gums &amp; mucilages</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-hexane</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Chloroform</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Acetone</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ethanol</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Water</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Anti Ulcer Activity

The rats were fasted for 48 h but allowed free access to water ad libitum. They were randomly selected and divided into five groups with six animals in each group. The study protocol was approved by institutional ethical committee. Group 1 received normal saline 10ml/kg body weight while group II received standard drug (Ranitidine30 mg/kg). Groups III, IV and V received 200, 400 and 800 mg/kg of the ethanolic extract, respectively, and all drugs were administered orally. One hour later, ulceration was induced by intragastric instillation of 0.5 ml of 90% ethanol and 1 h after ethanol administration, rats were anaesthetized using ether and the stomachs were removed and opened along the greater curvature to macroscopically examine any ulcerative lesions. The number, length and severity of the ulcers were noted and scored on a point scale.

The scores were as below:
0 = No lesion
0.5 = red coloration
1 = spot ulcer
1.5 = hemorrhagic streak
2 = ulcers more than 3 and less than 5mm
3 = perforations

Ulcerative index can be calculated by the following formula:

\[ \text{Ulcerative index} = \frac{(n \text{ lesion I})^2 + (n \text{ lesion II})^2 + (n \text{ lesion III})^2}{n \text{ animals}} \]

% ulcer inhibition = \[\frac{(\text{Control mean} - \text{test mean})}{\text{Control mean}} \times 100\]

Statistical Analysis:

All the values were expressed as Mean ± standard error mean (S.E.M) and analyzed for ANOVA and Dunnet’s – T test. Then the results obtained by this study were expressed in the table no.4.

RESULTS

Preparation of extract

The extractive values of Alangium salvifolium using soxhlation were given in table 1.

Identification Of Phyto Constituents

The phytoconstituents present in the different extracts were tabulated in the table 2

Pharmacological studies

Acute oral toxicity studies

From the table No.3 it can be concluded that there is no mortality and toxicity symptoms for the ethanolic extract of the plant. So the dose was optimized up to 1500mg/kg.

\[ \text{LD}_{50} = \text{higher dose} - \left( \frac{a \times b}{n} \right) \]

LD50 dose was optimized up to 1500mg/kg.

ED50 = LD50 / 10

Anti Ulcer Activity

By performing anti ulcer activity on male Wister rats, it can be concluded that the ethanolic extract of Alangium salvifolium has a significant anti ulcer activity at 400mg/kg and 800mg/kg dose. The results were tabulated in the table No. 4.

Table 4: Shows Antulcer Activity Of Ethanolic Extract Of Alangium Salvifolium

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment group</th>
<th>Dose (mg/kg)</th>
<th>Ulcer index (U.I.)</th>
<th>% Maximal protection of ulceration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (Saline)</td>
<td>10ml/kg</td>
<td>5.33 ± 0.33</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>Ranitidine (Standard)</td>
<td>30</td>
<td>0.33 ± 0.21</td>
<td>93.81*</td>
</tr>
<tr>
<td>3</td>
<td>Ethanolic extract of A. Salvifolium</td>
<td>200</td>
<td>3.17 ± 0.60</td>
<td>40.52*</td>
</tr>
<tr>
<td>4</td>
<td>Ethanolic extract of A. Salvifolium</td>
<td>400</td>
<td>1.00 ± 0.68</td>
<td>81.24*</td>
</tr>
<tr>
<td>5</td>
<td>Ethanolic extract of A. Salvifolium</td>
<td>800</td>
<td>0.50 ± 0.50</td>
<td>90.61*</td>
</tr>
</tbody>
</table>

All values are expressed as Mean ± SEM

*P < 0.01 as compared with control groups.

DISCUSSION

The phytoconstituents present in the plant were extracted by using different solvents of increasing polarity like n-hexane, chloroform, ethyl acetate, acetone, ethanol and water. The phytoconstituents were identified by various chemical tests which showed the presence of various phytoconstituents, glycosides, alkaloids, flavonoids, tannins, carbohydrates, steroids and terpenoids. The acute toxicity studies mainly aims at establishing the therapeutic index i.e., the ratio between the pharmacological effective dose and the lethal dose on the same strain and species (LD50/ED50). In any
screening program, acute toxicity on mice is usually performed first. This is a test in which a single dose of the drug was used in each animal on one occasion only for the determination of CD50 or median lethal dose i.e., the dose that kills 50% of the animals. CD50 value was determined by a 24 hour test using mice by the oral administration of the drug. The ethanolic extract of leaves of *Alangium salvifolium* was evaluated using mice. The LD50 was calculated by using karber’s method. The ED50 value was found to be 150 mg/kg body weight. By performing anti ulcer activity on male wistar rats, it can be concluded that the ethanolic extract of *Alangium salvifolium* has a significant anti ulcer activity at 400mg/kg and 800mg/kg dose that is in a dose dependent manner. The results were comparable with that of standard and control groups. The phytoconstituents present in ethanolic extract like flavonoids and phenolic compounds may be responsible for the said activity. Furthermore it is required to carry out detailed study on mechanism by which the said action was being shown and to isolate and purify the compounds responsible for the anti ulcer activity.

REFERENCES